Mitchell *et al.* have shown that a $|30,03\rangle$ state can be created by probabilistically superposing three photons with specific polarizations. The experiment closely follows the proposal by Hofmann[7] and is related to several other proposals[8–10]. An interference pattern with resolution $\lambda/3$ is obtained by detecting three-photon coincidences. Walther *et al.* present a variation of the $|40,04\rangle$ state. They show how double pairs of polarization-entangled photons emerging from two separate (but phase-related) sources can be combined to give a pure four-photon interference pattern. A clever choice for the detection of four-photon coincidences reveals the desired interference pattern with a resolution of $\lambda/4$.

Both schemes[2,3] have proved in an elegant way that the diffraction limit can be beaten, and both are in principle scalable to even higher resolutions. However, from a practical point of view, the signal-to-noise ratio of the interference fringes should also be analysed. Currently, it requires very long measurement times to resolve three- and, in particular, four-photon interference fringes with rather low signal-to-noise ratios. Single-photon interference fringes can quickly be detected with much higher signal-to-noise ratios,

resulting in higher accuracy for, for example, a position measurement. To push the 'high NOON' technology forward, it will be crucial to develop bright entangled multi-photon sources and efficient multi-photon detectors. Looking some years ahead, it doesn't seem unrealistic to expect commercial applications of entangled photons in secure data transmission systems, high-resolution optical read-out and storage systems, and perhaps even quantum computation. As if the world isn't entangled enough! ∎

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1. Dirac, P. A. M. *The Principles of Quantum Mechanics* (Clarendon, Oxford, 1982).
2. Mitchell, M. W., Lundeen, J. S. & Steinberg, A. M. *Nature* **429**, 161–164 (2004).
3. Walther, P. *et al. Nature* **429**, 158–161 (2004).
4. Ou, Z. Y., Zou, X. Y., Wang, L. J. & Mandel, L. *Phys. Rev. A* **42**, 2957–2965 (1990).
5. Rarity, J. G. *Phys. Rev. Lett.* **65**, 1348–1351 (1990).
6. Fonseca, E. J. S., Monken, C. H. & Padua, S. *Phys. Rev. Lett.* **82**, 2868–2871 (1999).
7. Hofmann, H. F. Preprint at http://arxiv.org/abs/quant-ph/0311198 (2003).
8. Pryde, G. J. & White, A. G. Preprint at http://arxiv.org/abs/quant-ph/0304135 (2003).
9. Kok, P., Lee, H. & Dowling, J. P. *Phys. Rev. A* **65**, 052104 (2002).
10. Fiurasek, J. *Phys. Rev. A* **65**, 053818 (2002).

**Membrane trafficking**

# Dual-key strategy

## Toshiki Itoh and Pietro De Camilli

Traffic flow between cellular compartments is controlled by recruitment of cytoplasmic proteins. New work exemplifies a dual-key mechanism, involving membrane lipids and proteins, that coordinates this control.

Our cells contain a series of distinct compartments that do different jobs and have different properties. The membranes that clad each of these compartments — like the plasma membrane that encases the cell — are defined by precise molecular compositions, which are preserved despite the continuous influx and efflux of components in transit to and from other cellular locations. Precision is the hallmark of this flow of traffic, too, which must be directed appropriately between compartments. All of this is achieved, in part, by the reversible recruitment of regulatory proteins from other parts of the cell to specific membranes or membrane regions. A growing amount of evidence hints that membrane lipids cooperate with membrane proteins to control this recruitment. In *Nature Cell Biology*, Godi and colleagues[1] reveal an example of this cooperative spirit, which helps to ensure the proper progression of cargo from one compartment — the *trans*-Golgi network (TGN) — to the cell surface.

Phosphatidylinositol is one of the most common lipids in cellular membranes, and

generally localizes to the cytosolic side of a membrane (the side that faces into the cell). The reversible addition of phosphate groups to (that is, phosphorylation of) the inositol ring of this lipid, at the 3, 4 or 5 positions, generates seven phosphoinositide species in mammalian cells. Each species recognizes, with varying affinities and specificities, certain modules or amino-acid motifs that are found in cytosolic proteins. So, in a mechanism reminiscent of the phosphorylation of tyrosine amino acids in membrane proteins[2], differential phosphorylation of the inositol ring helps to control the recruitment of specific proteins to membranes. In turn, the distribution of different phosphoinositides to different membranes depends on the distribution of the enzymes that add or remove phosphate groups[3,4].

Two of the proteins to which phosphoinositides bind are FAPP1 and FAPP2 (FAPP stands for 'four-phosphate-adaptor protein'). These are members of a protein family that is thought to function in the sorting or metabolism of lipids[5]. Both proteins have a so-called pleckstrin homology (PH) domain,
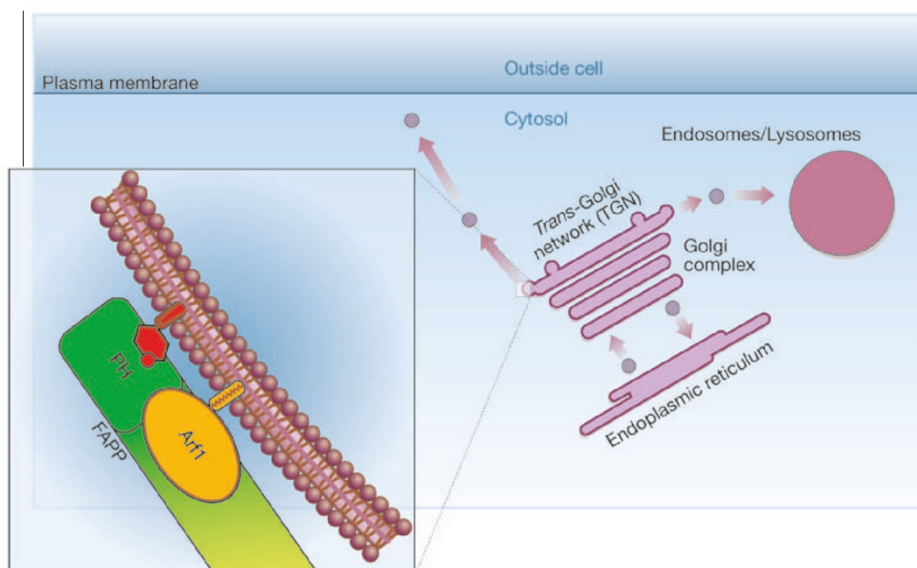
Figure 1 **Mechanism for the recruitment of the proteins FAPP1 and FAPP2 to the *trans*-Golgi network (TGN). Some of the main compartments within cells are shown, namely the endoplasmic reticulum, the Golgi complex (the part of the complex that is farthest from the endoplasmic reticulum being the TGN) and endosomes/lysosomes. Vesicles carry cargo from one compartment to another. Godi *et al.*[1] propose that the FAPPs participate in vesicular transport from the TGN to the cell surface. The binding of these proteins to TGN membranes is mediated by their pleckstrin homology (PH) domains (see inset), through the interaction of this domain with the lipid phosphatidylinositol-4-phosphate (red) and the protein Arf1. If one of these binding partners is missing, FAPPs no longer localize to the TGN.**

which in this case binds specifically to phosphatidylinositol-4-phosphate (PtdIns(4)P) — a phosphoinositide involved in the formation of membrane-clad organelles, such as vesicles and tubules, that carry cargo from the TGN[3,6–8].

Godi and co-workers[1] now show that both FAPP1 and FAPP2 are found at the TGN (Fig. 1). More notably, when the expression of these proteins is prevented, the transport of cargo from this compartment to the cell surface is impaired. Conversely, when cells are forced to produce too much of the isolated PH domain of these proteins, long, narrow tubules, which do not break off into vesicles, emerge from the TGN. These tubules contain cargo that is destined for the cell surface, and extend towards the plasma membrane but do not fuse with it. The authors interpret the appearance of these tubules as resulting from competition between the overexpressed PH domains and the cell's own PtdIns(4)P-binding proteins — possibly the FAPPs themselves — that participate in the creation and breaking-off of vesicles destined for the cell surface.

Godi *et al.* also show that the localization of the FAPPs to the Golgi complex is prevented by a mutation in the PH domain that stops it from binding to PtdIns(4)P, as well as by inhibiting the production of this phospholipid. Moreover, in agreement with previous studies[5], the authors find that the PH domain alone can localize to the TGN, indicating that this lipid-binding domain contains all the information needed for such localization.

How exactly the FAPPs assist in the formation of carriers destined for the cell surface remains uncertain, although the presence of a glycolipid-transfer domain in FAPP2 points to a potential link to lipid metabolism[1,5]. Regardless, Godi and colleagues' findings are significant because, until now, molecules that bind PtdIns(4)P and explain its previously discovered role of directing traffic from the Golgi complex to the cell surface[3,6,7] had not been identified. Another protein implicated in vesicular traffic at the TGN, clathrin adaptor-1 (AP-1), binds PtdIns(4)P (ref. 8) but does not participate in trafficking to the cell surface.

The new work also explains the very selective targeting of the FAPPs (and their PH domains) to the Golgi complex, despite the presence of PtdIns(4)P in other membranes. Previous studies[5,9] had shown that the PH domain of oxysterol-binding protein — a member of the same protein family as the FAPPs — binds to PtdIns(4)P and localizes mainly to the Golgi complex. But a mutation in this particular PH domain that prevented it from binding to the lipid did not stop it from being targeted to the TGN. In yeast, this lipid-independent localization required the function of Arf1, a protein of the small-GTPase family that localizes to Golgi membranes[5]. It remained unclear, however, whether this effect of Arf1 was direct or indirect.

Godi *et al.* show that the PH domains of the FAPPs bind directly to Arf1 as well as to PtdIns(4)P. Moreover, although a single

mutant PH domain that cannot bind to PtdIns(4)P no longer localizes to the Golgi complex, this localization is restored when the mutant PH domain is expressed as a dimer, because the two Arf1-binding sites from the two PH domains cooperate. Arf1 also interacts with, and recruits to the Golgi complex, an enzyme that phosphorylates the 4 position of phosphatidylinositol[10] — so Arf1 can lead to FAPP recruitment through at least two independent, but synergistic, mechanisms. Interestingly, the binding of AP-1 protein to membrane also requires Arf1 (ref. 11), hinting that PtdIns(4)P and Arf1 cooperate in several membrane-trafficking events at the TGN.

The discovery of protein modules that bind selectively to specific phosphoinositides has prompted the use of fluorescently tagged modules as 'reporters' of the distribution of the cognate phospholipids in living cells[12]. These probes have greatly advanced our understanding of phosphoinositide signalling. However, as Godi and colleagues' study shows[1], these modules might contain binding sites for membrane proteins as well, so one cannot rely on such probes to reveal the entire distribution of a given phosphoinositide. Dual interactions with phosphoinositides and other membrane determinants have been detected for other PH domains too[1,13]. Furthermore, a recent survey of all of the PH domains in budding yeast revealed that the subcellular targeting of these domains was only partly correlated with their phosphoinositide-binding specificities *in vitro*[14]. And similar dual interactions might apply to other phosphoinositide-binding modules as well, not just PH domains[15].

The interaction of cytosolic proteins with both lipids and proteins on a target membrane is an efficient dual-key strategy to control their recruitment to membranes. Only when both the lipid-binding and protein-binding sites are engaged is the interaction with the membrane strong enough. The two elements of the code can be controlled independently, affording the possibility of fine-tuning the spatial and temporal regulation of recruitment. Small GTPases and phosphoinositides have emerged as important switches in the regulation of membrane interfaces. This latest study[1] provides yet another example of how the two machineries cooperate with each other. ■

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1. Godi, A. *et al. Nature Cell Biol.* **6**, 393–404 (2004).
2. Schlessinger, J. *Cell* **103**, 211–225 (2000).
3. Odorizzi, G., Babst, M. & Emr, S. D. *Trends Biochem. Sci.* **25**, 229–235 (2000).
4. Wenk, M. R. & De Camilli, P. *Proc. Natl Acad. Sci. USA* (in the press).

5. Levine, T. P. & Munro, S. *Curr. Biol.* **12,** 695–704 (2002).
6. Walch-Solimena, C. & Novick, P. *Nature Cell Biol.* **1,** 523–525 (1999).
7. Hama, H. *et al. J. Biol. Chem.* **274,** 34294–34300 (1999).
8. Wang, Y. J. *et al. Cell* **114,** 299–310 (2003).
9. Levine, T. P. & Munro, S. *Curr. Biol.* **8,** 729–739 (1998).
10. Godi, A. *et al. Nature Cell Biol.* **1,** 280–287 (1999).
11. Stamnes, M. A. & Rothman, J. E. *Cell* **73,** 999–1005 (1993).
12. Balla, T. & Varnai, P. *Science STKE* doi:10.1126/stke.2002.125.pl3 (2002).
13. Varnai, P. *et al. J. Biol. Chem.* **277,** 27412–27422 (2002).
14. Yu, J. W. *et al. Mol. Cell* **13,** 677–688 (2004).
15. Chidambaram, S., Mullers, N., Wiederhold, K., Haucke, V. & von Mollard, G. F. *J. Biol. Chem.* **279,** 4175–4179 (2004).

## Physiology

# Orphan detectors of metabolism

Steven C. Hebert

There are myriad G-protein-coupled receptor proteins in living organisms, but the functions of many are unknown. Two of them are now shown to provide a link between metabolism and blood pressure.

One of the first things that biology students are taught is how cells liberate usable energy from food and oxygen. In eukaryotic cells (loosely speaking, those that have nuclei), much of this process of respiration occurs in double-membrane-bounded compartments known as mitochondria. A crucial stage in respiration is the tricarboxylic acid (TCA) cycle or Krebs cycle[1], into which are fed the products of the breakdown of sugars, fats and proteins, and out of which comes reducing power, used to generate the energy-storing molecule ATP. As testimony to the importance of this process, mutations in human genes encoding TCA-cycle enzymes have been associated with inherited cancers and severe generalized organ abnormalities[2]. It has been known for some time that TCA-cycle intermediates are also present outside cells, in the blood circulation, and that extracellular levels of these organic acids increase when tissues are damaged by low oxygen supplies or other 'insults'. On page 188 of this issue, He and colleagues[3] show, unexpectedly, that two such intermediates act as signalling molecules, linking the metabolism and injury of tissues with blood pressure.

Cells can detect and respond to a wide range of external signals by using receptor proteins on their surfaces. G-protein-coupled receptors (GPCRs) are the largest and most diverse group of such detectors; as their name suggests, they transmit extracellular information into cells by coupling to G proteins — common molecular switches — in the cytoplasm[4]. Although all GPCRs have similar structures, they show considerable diversity at the sequence level. However, on the basis of specific sequence motifs, they have been divided into three distinct families, each containing subfamilies that often have related stimuli (ligands)[5,6].

The number of GPCRs is growing continually: many new putative receptor genes have been identified in genome databases by computer-based screening for similar sequences. But for many of the newly discovered receptors, a physiologically relevant ligand is unknown[6–8]. These 'orphan' receptors are of considerable interest to biologists because they might define new ways in which cells can respond to their external environment, and to the pharmaceutical industry because they might provide new targets for drug development. Finding the relevant ligand for an orphan receptor can be a daunting task, however. One approach has been to use phylogenetic analyses — based on similarities in amino-acid sequence — to place newly identified receptors into one of the GPCR subfamilies, in an attempt to identify the ligand by the company the receptor keeps. But this is frequently not successful, and finding the correct ligand often requires a wide-ranging screening approach.

This was just the case for the orphan receptors GPR91 and GPR99. A phylogenetic approach had hinted that these proteins might bind nucleotides, given their membership in the P2 purinoceptor subfamily. He *et al.*[3] have now found, however, that nucleotides do not activate these receptors. Instead, the TCA-cycle intermediates α-ketoglutarate and succinate are physiologically relevant ligands for GPR99 and GRP91 respectively.

What might these metabolic detectors do? Kidneys express both GPR91 and GPR99 (refs 7, 8), and He *et al.* provide compelling evidence that, via GPR91, succinate stimulates the release of renin from the kidneys. This reveals the mechanism behind the earlier observation that succinate increases the release of renin from isolated kidney capillaries[9]. Renin is part of the renin–angiotensin system (RAS) — a series of enzymes and molecules that culminate in the production of the eight-amino-acid peptide angiotensin II. This molecule in turn acts on blood vessels, causing them to constrict and so raising blood pressure.

Physiologically speaking, this could all be very useful. Mitochondrial dysfunction, caused by, for instance, an imbalance between energy demand and the food and oxygen
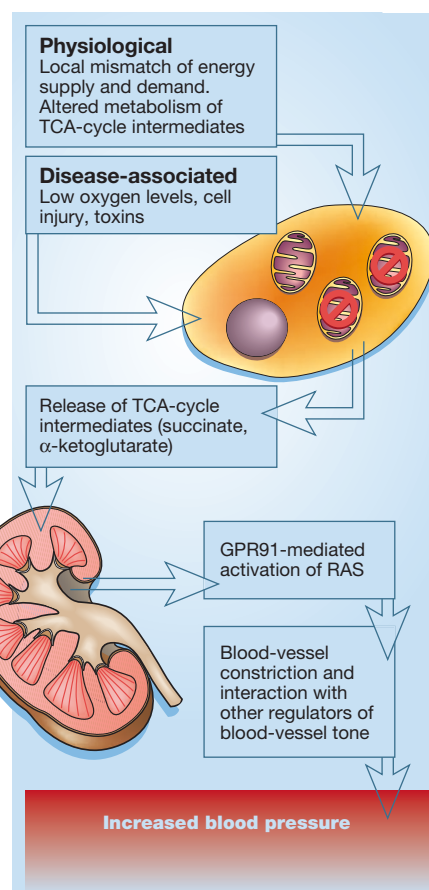


Figure 1 **Connecting metabolism to blood supply.** He *et al.*[3] have found that the 'orphan' G-protein-coupled receptors GPR91 and GPR99 detect succinate and α-ketoglutarate — intermediates produced by the tricarboxylic acid (TCA) cycle during respiration. They also find that succinate increases blood pressure in mice. The figure shows how this happens. A local mismatch of energy supply and demand, altered metabolism of TCA-cycle intermediates, or injury leads to mitochondrial dysfunction and the release of succinate and α-ketoglutarate. These molecules activate receptors in the kidney, causing the release of renin and activation of the renin–angiotensin system (RAS). The RAS leads to an increase in blood pressure and altered local blood flow. Physiologically, this system might act to regulate local blood flow to match metabolic demands. However, it might also result in hypertension or alter cellular function.

supply, increases the extracellular concentrations of TCA-cycle intermediates (Fig. 1). He and colleagues' findings show that these intermediates can then influence the RAS, possibly (among other effects) reducing blood flow in the kidneys. Kidneys receive more than their fair share of the cardiac output of blood (about 20%), so reductions in kidney blood flow provide a means of redirecting blood to other organs during crises.

On the other hand, the balance can also be tipped towards disease: He *et al.* found that treating mice with succinate led to high